Introduction of a New Housekeeping Gene Sequences for Rapid Identification of *Vibrio cholerae* Strains in the Clinical Samples

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Background: *Vibrio cholerae* is a noninvasive, gram-negative bacterium responsible for severe epidemics of cholera and endemic diarrhea in many parts of the world, especially developing countries. *Vibrio cholerae* strains may produce a variety of atypical biochemical reactions which may pose some difficulties in rapid identification and recovery of these bacteria from mixed samples isolated from clinical or environmental sources. To best of our knowledge *dna*E gene is widespread among all *Vibrio cholerae* strains. Our study is aimed at developing the use of PCR amplification of *dna*E housekeeping gene as a screening tool for identification of *Vibrio cholerae* among mixed clinical samples and comparison with the time consuming conventional biochemical methods.

Methods: A total of 24 *Vibrio cholerae* isolates of clinical origin isolated and subjected to serogrouping and subsequent PCR analysis using primers which specifically chosen to amplify within the *dna*E gene in the genome of the *Vibrio cholerae* isolates. *Vibrio cholerae* ATCC 14035 and *Pseudomonas aeruginosa* ATCC 27853 were used as positive and negative controls in each assay

Results: Among 24 *Vibrio cholerae* isolated, 21% identified as Ogawa and the remaining 79% as Inaba serogroup. PCR analysis of the *dna*E housekeeping gene revealed the presence of this gene in 100% of isolates examined and approximately 300 bp product was obtained from the isolates. The identity of the product was further confirmed using RFLP assay

Conclusions: The result of this study suggest the use of *dna*E housekeeping gene as a molecular marker for rapid identification of *Vibrio cholerae* in mixed clinical samples.

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Bacterial DNA Load in Cerebrospinal Fluid During Treatment of Bacterial Meningitis

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Background: Bacterial meningitis is an important cause of morbidity and mortality in Vietnam. The diagnosis can be difficult to establish, e.g. due to frequent pretreatment with antimicrobial agents. We developed a quantitative real-time PCR (qRT-PCR) to determine the bacterial DNA load in CSF during treatment of four major pathogens causing bacterial meningitis in Vietnam.

*Methods:*CSF samples were collected from 445 patients with suspected bacterial meningitis on hospital admission, after 48 hours, and 6—15 days after admission. Primers (Nm) were as described previously by Corless et al. Primers and probe for *Streptococcus suis* type 2 (SS2) detection were designed using Primer Express. PCR fragments were cloned in TA cloning vector. Serial 10 fold-dilutions of purified plasmid served as external standards for real-time quantitation

*Results:*SS2 was detected in 149 (33.5%) of samples collected on admission, Sp in 79 (17.8%), Nm in 29 (6.5%) and Hib in 5 (1.1%) using RT-PCR. The median bacterial DNA load on admission was 1.48×10^6 DNA copies/ml (range 1.0×10^3—1.1×10^8) for SS2, 4.98×10^5 DNA copies/ml (range 1.0×10^3—9.1×10^9) for Sp, 3.37×10^6 DNA copies/ml (range 1.0×10^3—1.13×10^8) for Nm. Clearance of bacterial DNA was gradual. Median bacterial DNA loads of SS2, SP and Nm were similar at each of the sampling times. DNA was still detectable in 69/156 (44.2%) samples collected 6—10 days after start of treatment and in 17/53 (32.07%) samples collected after 11—15 days

*Conclusions:*Median bacterial DNA loads are similar in meningitis caused by SS2, Sp and Nm but are highly variable when comparing individual patients. DNA loads decline only gradually during treatment, enabling diagnosis by PCR even after start of antimicrobial treatment. qRT-PCR can be used to study the association between bacterial DNA load and clearance and outcome.

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A Comparative Western Immunoblot Antibody Titration Test for Evaluation of *Bartonella* Therapy

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Introduction: Bartonella are zoonotic pathogens mainly transmitted by cats and occasionally dogs. They are difficult to isolate and most *Bartonella* infections of animals or people are diagnosed by detection of coexisting antibody (IgG).

Methods: We developed a single dilution western immunoblot (WB) for detection of *Bartonella* antibodies (IgG 1:100) for determination of *Bartonella spp.* infection. We adapted this test in a comparative titration format (pre and 6 months post therapy) to assess the elimination of *Bartonella* from cats, dogs and people

Results: Bartonella was isolated from 7 seropositive pet cats before doxycycline therapy. All 7 cats were culture negative 1 month post therapy but at 6 months only 5 of the 7 cats remained culture negative. All 5 of the culture negative cats had a titer decrease of 4 fold ($n = 4$) and 2 fold ($n = 1$) whereas the 2 cats where *Bartonella* was isolated 6 months post therapy had no titer decrease. In contrast, there was no titer decrease in 21 untreated *Bartonella*-seropositive pet cats observed for more than 1 year. 9,782 titrations were performed on *Bartonella*-seropositive pet cats 6 months after treatment with: azithromycin ($n = 9,162$), rifampin ($n = 469$) and doxycycline ($n = 151$). A 2 fold titer decrease occurred in 1,119 cats (11.4%) and a 4 fold or greater titer decrease occurred in 7,583 cats (76.5%). Thus, post ther-